

This Page Is Inserted by IFW Operations
and is not a part of the Official Record

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

**As rescanning documents *will not* correct images,
please do not report the images to the
Image Problem Mailbox.**

C1
CON't.

selected from the group consisting of: D405, Y410, T542, D543, K593, Y595, Y385, G387, and G388.

2. The enzyme mixture of claim 1, wherein said first enzyme is a DNA polymerase or a reverse transcriptase.
3. The enzyme mixture of claim 2, wherein said DNA polymerase is selected from the group consisting of: Taq DNA polymerase, Tth DNA polymerase, UlTma DNA polymerase, Tli DNA polymerase, Pfu DNA polymerase, KOD DNA polymerase, JDF-3 DNA polymerase, PGB-D DNA polymerase and DP1/DP2 DNA polymerase.
6. (Amended) An enzyme mixture comprising a first enzyme and a second enzyme, wherein said first enzyme is a wild type Pfu DNA polymerase, said second enzyme is a mutant Pfu DNA polymerase comprising a 3'-5' exonuclease activity and a reduced DNA polymerization activity.]
9. (Amended) The enzyme mixture of claim 6, wherein said mutant Pfu DNA polymerase comprises one or more mutations at amino acid positions selected from the group consisting of: D405, Y410, T542, D543, K593, Y595, Y385, G387, and G388.
10. (Amended) The enzyme mixture of claim 1 or 9, wherein said mutant Pfu DNA polymerase comprises one or more mutations selected from the group consisting of: D405E, Y410F, T542P, D543G, K593T, Y595S, Y385Q, Y385S, Y385N, Y385L, Y385H, G387S, G387P, and G388P.
11. (Amended) The enzyme mixture of claim 1, further comprising a PCR enhancing factor and/or an additive.
12. (Amended) The enzyme mixture of claim 6, wherein said mutant Pfu DNA polymerase comprises a mutation in its partitioning domain or the polymerase domain. ~
13. (Amended) A kit comprising a first enzyme, [and] a second enzyme, and packaging material therefor, wherein said first enzyme comprises a DNA polymerization activity, said second enzyme is a mutant Pfu DNA polymerase comprising one or more mutations at amino

C 3 acid positions selected from the group consisting of: D405, Y410, T542, D543, K593, Y595, Y385, G387, and G388.

CON't. 14. (Amended) The kit of claim 13, wherein said first enzyme is a DNA polymerase or a reverse transcriptase.

15. The kit of claim 14, wherein said DNA polymerase is selected from the group consisting of: Taq DNA polymerase, Tth DNA polymerase, U1Tma DNA polymerase, Tli DNA polymerase, Pfu DNA polymerase, KOD DNA polymerase, JDF-3 DNA polymerase, PGB-D DNA polymerase and DP1/DP2 DNA polymerase.

19. A kit comprising an enzyme mixture for DNA synthesis, said kit comprises a first enzyme and a second enzyme, and packaging material therefore, wherein said first enzyme is a wild type Pfu DNA polymerase, said second enzyme is a mutant Pfu DNA polymerase comprising a 3'-5' exonuclease activity and a reduced DNA polymerization activity.

C 4 21. (Amended) The kit of claim 13 or 19, further comprising one or more components selected from the group consisting of: a deoxynucleotide, a reaction buffer, a PCR enhancing factor and/or an additive, a control DNA template and a control primer.

C 22. (Amended) The kit of claim 19, wherein said mutant Pfu DNA polymerase comprises one or more mutations at amino acid positions selected from the group consisting of: D405, Y410, T542, D543, K593, Y595, Y385, G387, and G388.

23. (Amended) The kit of claim 13 or 22, wherein said mutant Pfu DNA polymerase comprises one or more mutations selected from the group consisting of: D405E, Y410F, T542P, D543G, K593T, Y595S, Y385Q, Y385S, Y385N, Y385L, Y385H, G387S, G387P, and G388P.

24. (Amended) A method for DNA synthesis comprising:

(a) providing an enzyme mixture, said enzyme mixture comprising a first enzyme comprising a DNA polymerization activity, and a second enzyme which is a mutant Pfu DNA polymerase comprising one or more mutations at amino acid positions selected from the group consisting of: D405, Y410, T542, D543, K593, Y595, Y385, G387, and G388; and

C4
Cont'd.

(b) contacting said enzyme mixture with a nucleic acid template, wherein said enzyme mixture permits DNA synthesis.

25. (Amended) The method of claim 24, wherein said nucleic acid template is a DNA molecule.

26. The method of claim 25, wherein said first enzyme is a DNA polymerase or a reverse transcriptase.

27. The method of claim 26, wherein said DNA polymerase is selected from the group consisting of: Taq DNA polymerase, Tth DNA polymerase, Ultma DNA polymerase, Tli DNA polymerase, Pfu DNA polymerase, KOD DNA polymerase, JDF-3 DNA polymerase, PGB-D DNA polymerase and DP1/DP2 DNA polymerase.

31. A method for DNA synthesis comprising:

(a) providing an enzyme mixture, said enzyme mixture comprising a wild type Pfu DNA polymerase as a first enzyme, and a mutant Pfu DNA polymerase as a second enzyme which comprises a 3'-5' exonuclease activity and a reduced DNA polymerization activity; and

(b) contacting said enzyme mixture with a nucleic acid template, wherein said enzyme mixture permits DNA synthesis.

32. A method for TA cloning of DNA synthesis product comprising:

(a) providing an enzyme mixture, said enzyme mixture comprising a Taq DNA polymerase as a first enzyme, and a mutant Pfu DNA polymerase as a second enzyme which comprises a 3'-5' exonuclease activity and a reduced DNA polymerization activity;

(b) contacting said enzyme mixture with a nucleic acid template, wherein said enzyme mixture permits DNA synthesis to generate a synthesized DNA product; and

(c) inserting said synthesized DNA product into a TA cloning vector.

C5

33. (Amended) The method of claim 31, or 32, wherein said mutant Pfu DNA polymerase comprises one or more mutations at amino acid positions selected from the group consisting of: D405, Y410, T542, D543, K593, Y595, Y385, G387, and G388.

34. (Amended) The method of claim 24, wherein said mutant Pfu DNA polymerase comprises one or more mutations selected from the group consisting of: D405E, Y410F, T542P, D543G, K593T, Y595S, Y385Q, Y385S, Y385N, Y385L, Y385H, G387S, G387P, and G388P.

35. The method of claim 24, 31 or 32, wherein said reaction mixture further comprises a PCR enhancing factor and/or an additive.

40. A mutant Pfu DNA polymerase with reduced DNA polymerization activity, wherein said mutant Pfu DNA polymerase comprises one or more mutations at amino acid positions selected from the group consisting of: T542, D543, K593, Y595, Y385, G387, and G388.

41. The mutant DNA polymerase of claim 40, wherein said mutant Pfu DNA polymerase comprises one or more mutations selected from the group consisting of: T542P, D543G, K593T, Y595S, Y385Q, Y385S, Y385N, Y385L, Y385H, G387S, G387P, and G388P.

47. A composition comprising a mutant Pfu DNA polymerase, wherein said mutant DNA polymerase comprises one or more mutations at amino acid positions selected from the group consisting of: T542, D543, K593, Y595, Y385, G387, and G388.

48. The composition of claim 47, wherein said mutant Pfu DNA polymerase comprises one or more mutations selected from the group consisting of: T542P, D543G, K593T, Y595S, Y385Q, Y385S, Y385N, Y385L, Y385H, G387S, G387P, and G388P.

54. A kit comprising a mutant DNA polymerase which comprises a reduced DNA polymerization activity and packaging material therefor, wherein said mutant Pfu DNA polymerase comprises one or more mutations at amino acid positions selected from the group consisting of: T542, D543, K593, Y595, Y385, G387, and G388.

55. The kit of claim 54, wherein said mutant Pfu DNA polymerase comprises one or more mutations selected from the group consisting of: T542P, D543G, K593T, Y595S, Y385Q, Y385S, Y385N, Y385L, Y385H, G387S, G387P, and G388P.

57. A mutant Pfu DNA polymerase produced by introducing a mutation in to a polynucleotide encoding a wild type Pfu DNA polymerase to produce a mutant Pfu DNA polymerase comprising one or more mutations at amino acid positions selected from the group consisting of: T542, D543, K593, Y595, Y385, G387, and G388.

58. A mutant Pfu DNA polymerase comprising a reduced DNA polymerization activity, wherein said mutant Pfu DNA polymerase is produced by the steps:

(a) providing a polynucleotide encoding a wild-type Pfu DNA polymerase;

(b) introducing one or more nucleotide mutations into said polynucleotide to produce a mutant polynucleotide encoding said mutant Pfu DNA polymerase; and

(c) expressing said mutant polynucleotide to produce said mutant Pfu DNA polymerase, wherein said mutant Pfu DNA polymerase comprises one or more mutations at amino acid positions selected from the group consisting of: T542, D543, K593, Y595, Y385, G387, and G388.

59. The mutant DNA polymerase of claim 58, wherein said mutant Pfu DNA polymerase comprises one or more mutations selected from the group consisting of: T542P, D543G, K593T, Y595S, Y385Q, Y385S, Y385N, Y385L, Y385H, G387S, G387P, and G388P.

60. A composition comprising a mutant Pfu DNA polymerase produced by expressing a polynucleotide encoding a Pfu DNA polymerase with a reduced DNA polymerization activity, wherein said mutant Pfu DNA polymerase comprises one or more mutations at amino acid positions selected from the group consisting of: T542, D543, K593, Y595, Y385, G387, and G388.

61. A composition comprising a mutant Pfu DNA polymerase comprising a reduced DNA polymerization activity, wherein said mutant Pfu DNA polymerase is produced by the steps:

(a) introducing a mutation into a polynucleotide encoding a wild-type Pfu DNA polymerase to produce said mutant Pfu DNA polymerase comprising one or more mutations at amino acid positions selected from the group consisting of: T542, D543, K593, Y595, Y385, G387, and G388;

(c) expressing said mutant polynucleotide to produce said composition comprising said mutant Pfu DNA polymerase.